Fig. 1

O1

	853 ... 860 ... 870 ... 880
LL44, LB68	..T G T T T A C T A A A A A T A T T T T G G T A A A G C A
N62, LB10	..T G T T T A C T A A A A G T A T T T T G G T A A A A C A
N141	..T G G C G A C T A A A A G T A T T T T G G T A A A A C A
	ISL7
N299	..A A A T T A C T A A A A A T A T T T T A G T A A A A C A
	ISL5

O2

	890 ... 900
LL44, LB68	T C T T G A T T T G T T T A G T A A A C G G G T C T A T A...
N62, LB10	T C T T G A T T T G T T T A G T A A A C A A G T C T A T A...
N141	T C T T G A T T T G T T T A G T A A A C A A G T C T A T A...
N299	T C T T G G T T T A T T T A G T A A A C A A G T C T A T A...

Comparison of *L. delbrueckii* operators sequences (O1 and O2). Arrows are for inverted repeats. The LL44 sequence is numbered according to figure 1. Sequence of the second helix of *lacR* (repressor) is indicated.

Fig. 2

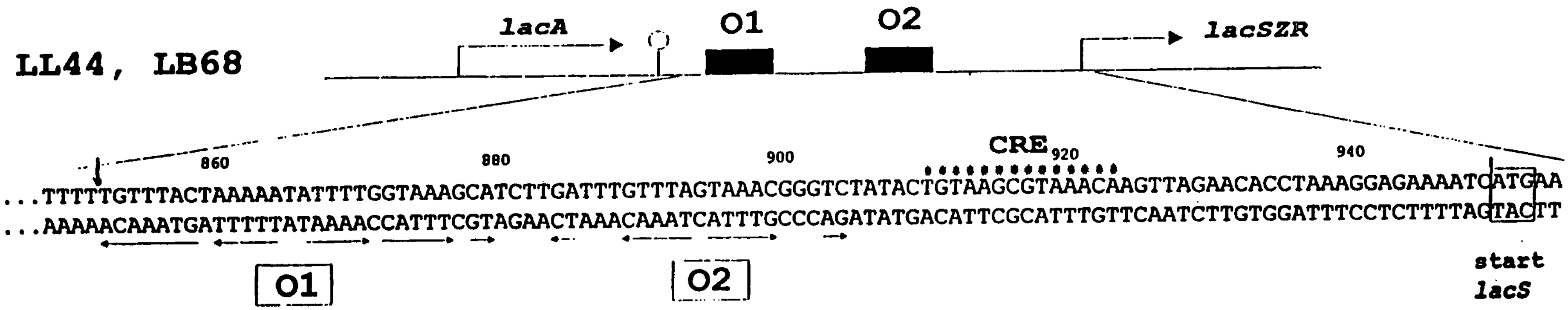

Fig. 3

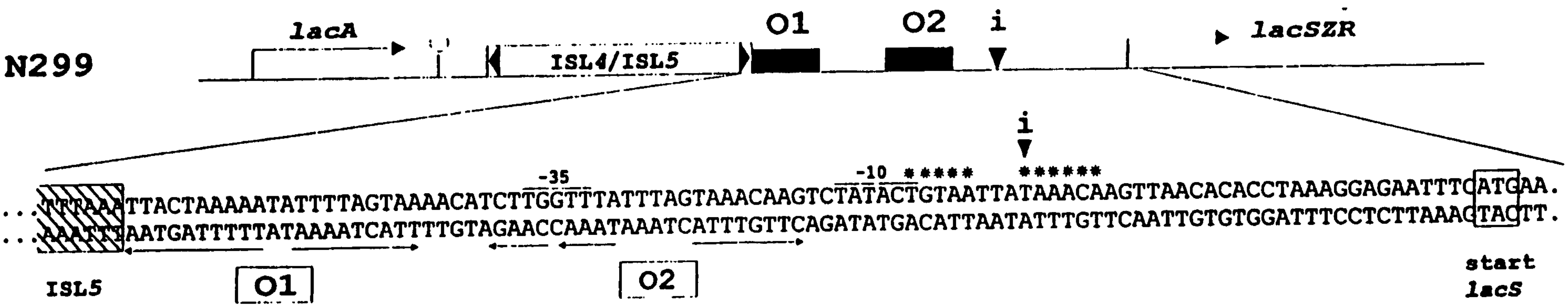

Fig. 2 u. 3: Organisation of the promoter region of LL44 and N299 *lac* operon. OperatorsO1 and O2 are indicated by black boxes. The inverted repeats of the operators are represented by arrows. The sequence responsible for catabolite repression (CRE) is overdrawn by stars.
The inverted repeat of ISL5 is boxed and shaded. The initiation of transcription is shown by an i (arrow head) (Leong-Morgenthaler et al, 1991). The promoter sequence of LL44 is numbered according to figure 1. The picture is not drawn to scale.

```
1     GAATTTTGTCTGGATGCTCAGGAAGCCCGCCAGCTCAAGCTGGTGATTCAGCCACTTTTT
            stop lacZ                                      RBS
61    ACTGAATAATGCTACAATTGACTTAACAGCATAAAATTTTAGTAAAAGCGAGTGAAGAAG

121   ATGGCAACGATCAGAGAAGTGGCCAAGGCAGCCGGCGTGTCGCCAGCGACGGTTTCCCGG
1     M  A  T  I  R  E  V  A  K  A  A  G  V  S  P  A  T  V  S  R
                      helix             turn         helix
181   GTCTTGAACTATGACCAGACCCTGTCGGTCAATGAGGCAACGCGGCAGAAGATATTCAAA
21    V  L  N  Y  D  Q  T  L  S  V  N  E  A  T  R  Q  K  I  F  K

241   ACTGCTGAAGCCATGCACTACCATAAGAGCCGGAAGACCAGAAAGAGCAAGCAAAAGCGC
41    T  A  E  A  M  H  Y  H  K  S  R  K  T  R  K  S  K  Q  K  R
301   CTGGCGATCTGCCTGTGGTGTGACCAAGACCAGGAGATCAAGGACCTCTATTACTATTCA
61    L  A  I  C  L  W  C  D  Q  D  Q  E  I  K  D  L  Y  Y  Y  S
361   ATCAGAACCAGCGCGCAAGCAGAGGCCAAGAAGCAGGGACTTGAAAGCCAGGTCATTTAT
81    I  R  T  S  A  Q  A  E  A  K  K  Q  G  L  E  S  Q  V  I  Y
421   CCGGCTGATCCTTTGCCCGATCCAGCTGCTTTAAGCGGGATTATCATGATTGGCTACCAG
101   P  A  D  P  L  P  D  P  A  A  L  S  G  I  I  M  I  G  Y  Q
481   CAGTATTCGCCAGACCGCTTGAATGAAGTCAAAAAGTCTGGCCTGCCCCTGGTCTTTGTC
121   Q  Y  S  P  D  R  L  N  E  V  K  K  S  G  L  P  L  V  F  V
541   GATACTGACACCTTAAAATTGGGTTACTGCTCAGTTGTGGCTGACTTTGGCCAGGCCATG
141   D  T  D  T  L  K  L  G  Y  C  S  V  V  A  D  F  G  Q  A  M
601   CAGGAGGCGCTAGAGGTCTTCTGGGGGCAGGGCAGGGAGCGGATCGCCCTTTTGGATGGT
161   Q  E  A  L  E  V  F  W  G  Q  G  R  E  R  I  A  L  L  D  G
661   GATTTGGACAGTAATTTTGATAAAAACAACTTGGTCGACTTCCGCTTCCGCGATTATAAG
181   D  L  D  S  N  F  D  K  N  N  L  V  D  F  R  F  R  D  Y  K
      ▼
721   AAGAGCCTCGCGGCCCGCGGCCAGTACGACCCGGACTTAGTCTATGTTGGAAACTTCACT
201   K  S  L  A  A  R  G  Q  Y  D  P  D  L  V  Y  V  G  N  F  T
781   CCGCAATCTGGCTATGAAGCCATTAAAGAAGCTCTTAAGTCCGGCTCCTTCCCGAAAGCC
221   P  Q  S  G  Y  E  A  I  K  E  A  L  K  S  G  S  F  P  K  A
841   TTGATTGCGGCTAATGACGCCATGGCTATTGGAGCATTGAAGGCCTTTAAAGAAGCTGGA
241   L  I  A  A  N  D  A  M  A  I  G  A  L  K  A  F  K  E  A  G
901   ATTAAAGTCCCAGAGGACGTCAGTCTGATTTCTTTTAATGACACAACGGCAGCAGAATTT
261   I  K  V  P  E  D  V  S  L  I  S  F  N  D  T  T  A  A  E  F
961   GCCAACCCAGCCTTGACTAGCGTACATGTAGAGACCCAGCAGATGGGCCGAGCCAGCGTC
281   A  N  P  A  L  T  S  V  H  V  E  T  Q  Q  M  G  R  A  S  V
1021  AAGGTCATGAAAGACCTGCTGGATGATGATGAAGCCGGCACTTACAAGGTCACTTTCCCA
301   K  V  M  K  D  L  L  D  D  D  E  A  G  T  Y  K  V  T  F  P
1081  ACAAAACTCGTTTACCGGGAATCTTGCCCAAAAGCATAAGGGCATAGAGCATAATAACAG
321   T  K  L  V  Y  R  E  S  C  P  K  A  *

1141  CAAAGAAATAGCTTGGAGATTGATTTCTCCAAGCTATTTTTCGTATATATTATGGCTGC
                                                    stop asnA

1201  ATTCTGTTGATCATTCTTGGGAATGGGACAGCTTCACGAACGTGGTCCAGCTTGCAGATC
1261  CAGGCAATGACCCGTTCAAAG
```

Figure 4:

Nucleotide and amino acid sequences of the *L. delbrueckii* subsp. *lactis* LL44 *lacR* gene. Start (121) and stop (1119) codons are boxed. Putative *lacR* RBS is underlined. The putative rho-independent terminator is underlined by convergent arrows. Stop codons of the beta-galactosidase (*lacZ*) and Asn t-RNA synthetase (*asnA*) genes are boxed. Insertion sequence of ISL*3* is represented by a large open arrow. Single base pair deletion (722) in the mutant LZL102 is shown by an arrow head, leading to a premature stop codon (758) underlined.

LL44
LB68*

N62

N141

N299

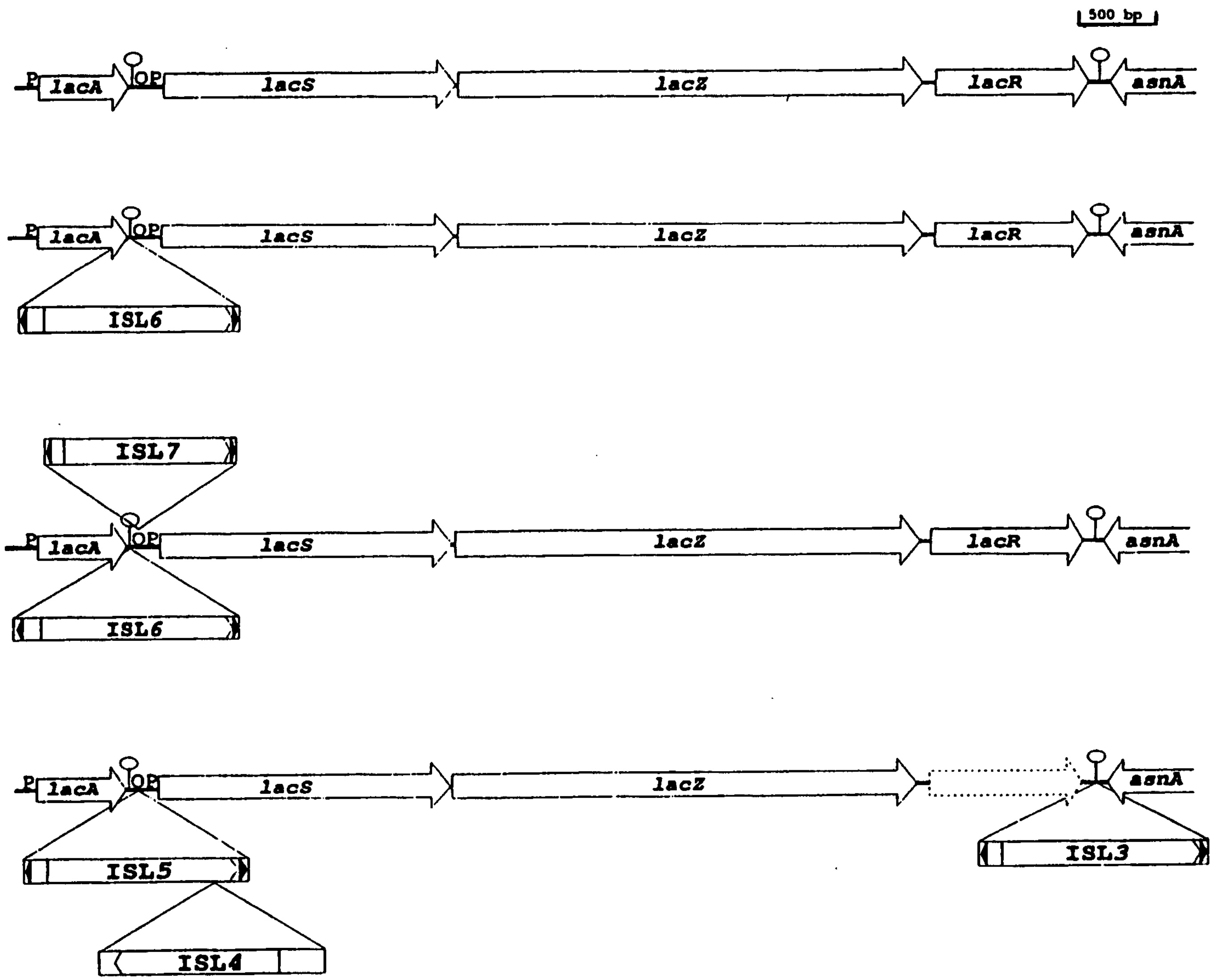

Figure 5 :

Physical map of the lactose operon of the different *L. delbrueckii* studied. Open arrows are for the *lac* operon genes and dashed arrow is for inactivated *lacR*. Boxes are for the different IS-elements, where the arrows heads are for the inverted repeats.
* same sequence as LL44 except an insertion in the 5' end of the *lacA* gene.

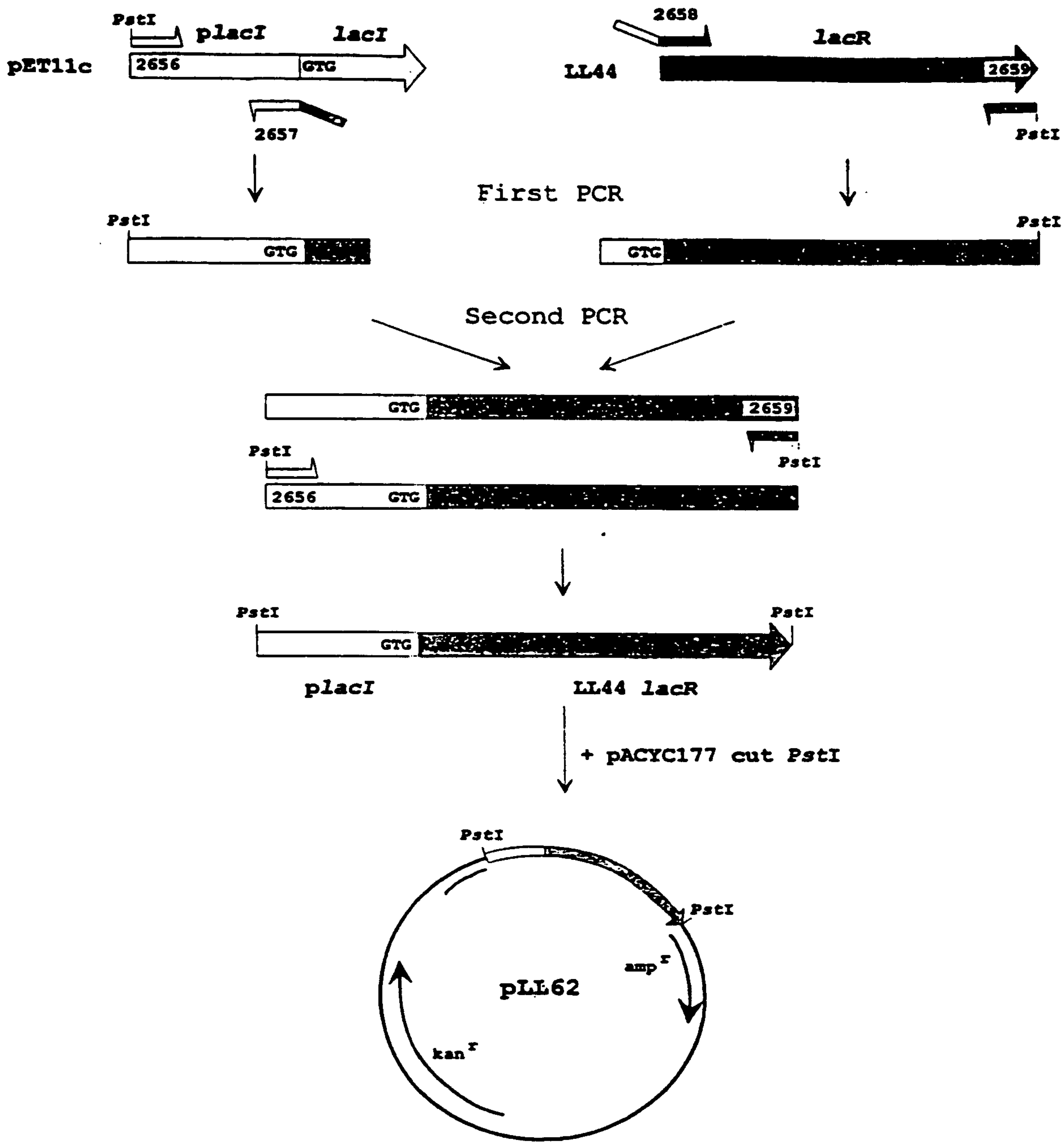

Figure 6 : Schematic representation of the construction of a pLL62. The dark box is for LL44 *lacR* gene and the white box is for the promoter region of the *lacI* gene of pET11c. Both were linked by PCR amplification using the SOEing method.

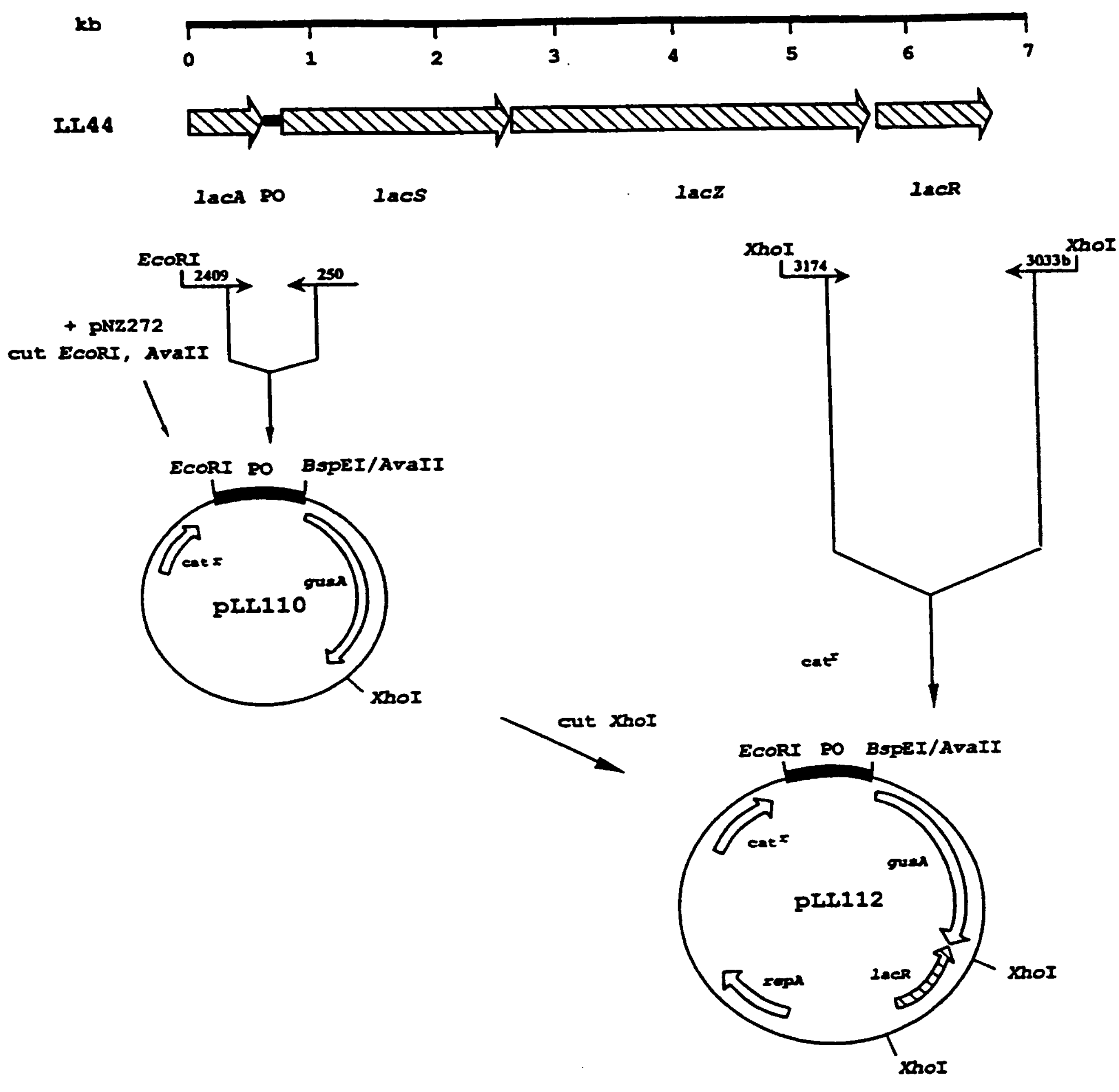

Figure 7 : Schematic representation of the construction of pLL110 and pLL112. Dashed arrows are for the genes of the *L. delbrueckii lac* operon, and open arrows for plasmid genes. The dark box is for the promoter region cloned in front of the *gusA* gene. Plasmids are not drawn to scale. The simple arrows represent the primers used to amplify the cloned regions.

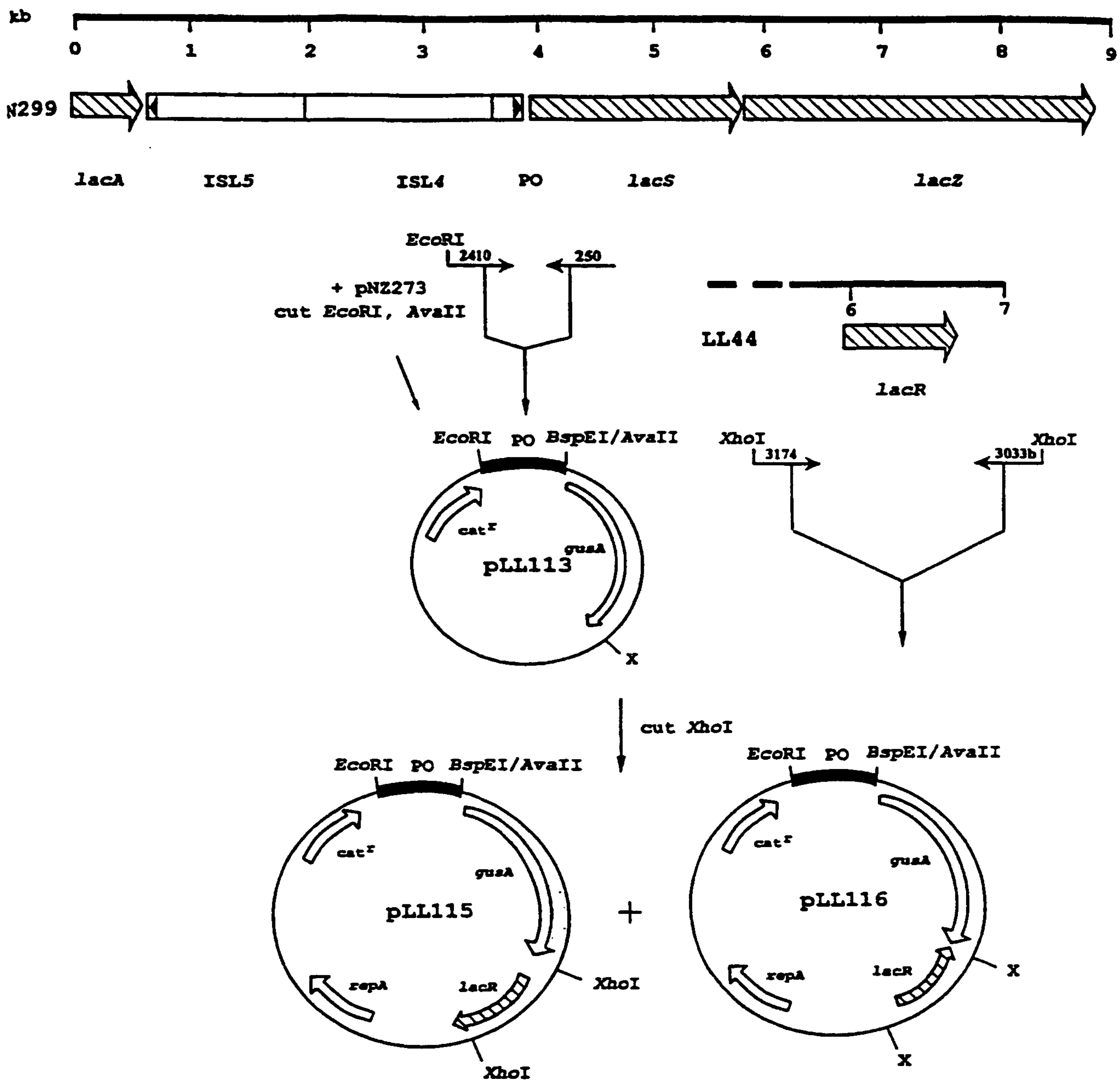

Figure 8 : Schematic representation of the construction of pLL113, pLL115 and pLL116. Dashed arows are for the genes of the *L. delbrueckii lac* operon, and open arrows for plasmid genes. The open box containing arrow heads represents the IS-elements. The dark box is for the promoter region cloned in front of the *gusA* gene. Plasmids are not drawn to scale. The simple arrows represent the primers used to amplify the cloned regions.

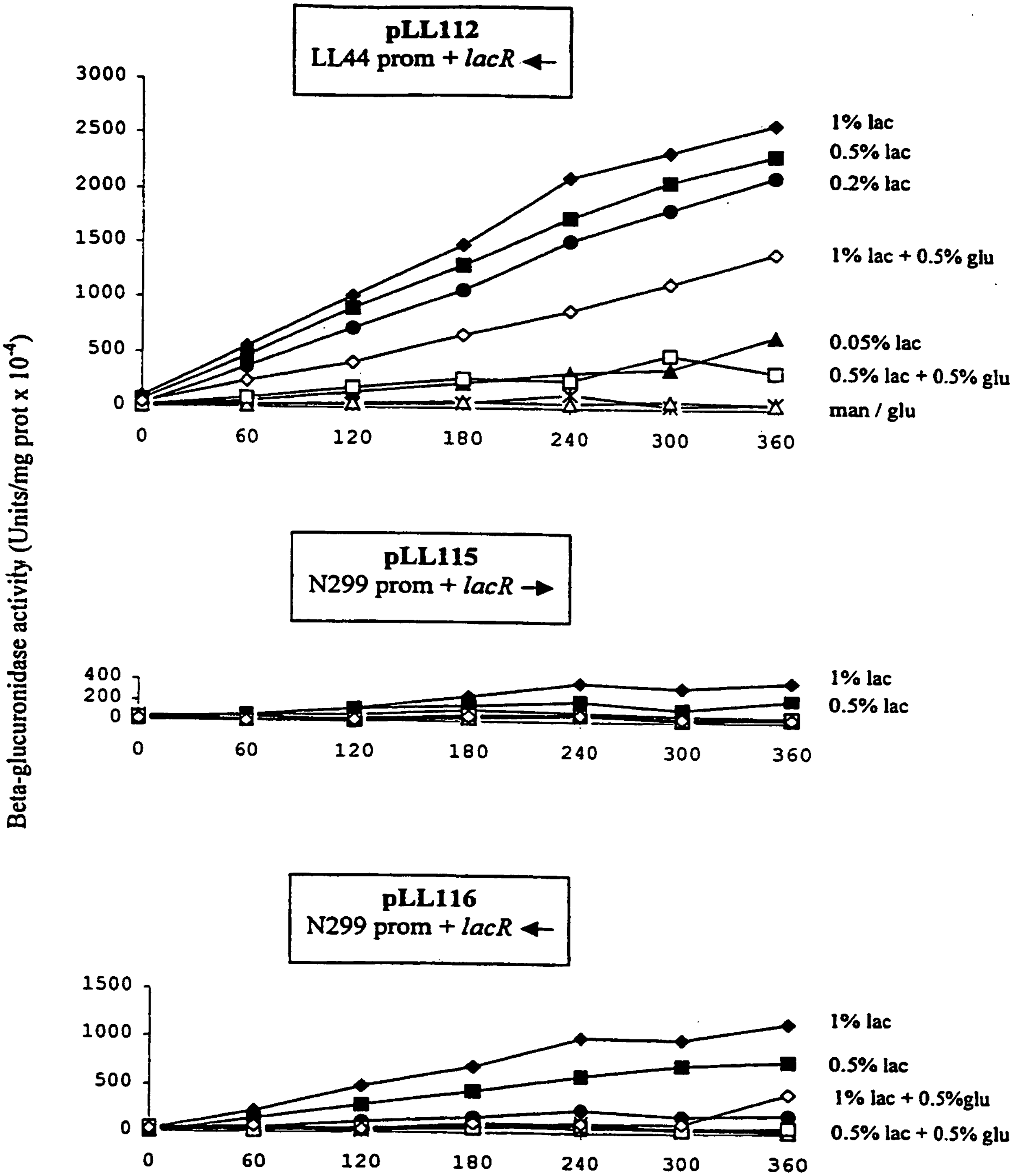

Figure 9 : Beta-glucuronidase activity (mean of three experiments) of *Lactococcus lactis* MG1363 containing different *Lactobacillus delbrueckii lac* promoter and the *lacR* gene of LL44. The *lacR* orientation compared to the *gusA* gene is represented by an arrow. The medium used was M17 containing : 0.5% mannose(✕), 0.05% lactose (▲), 0.2% lactose (●), 0.5% lactose (■), 1.0% lactose (◆), 0.5% glucose (△),0.5% glucose + 0.5% lactose (□) and 0.5% glucose + 1.0% lactose (◇).

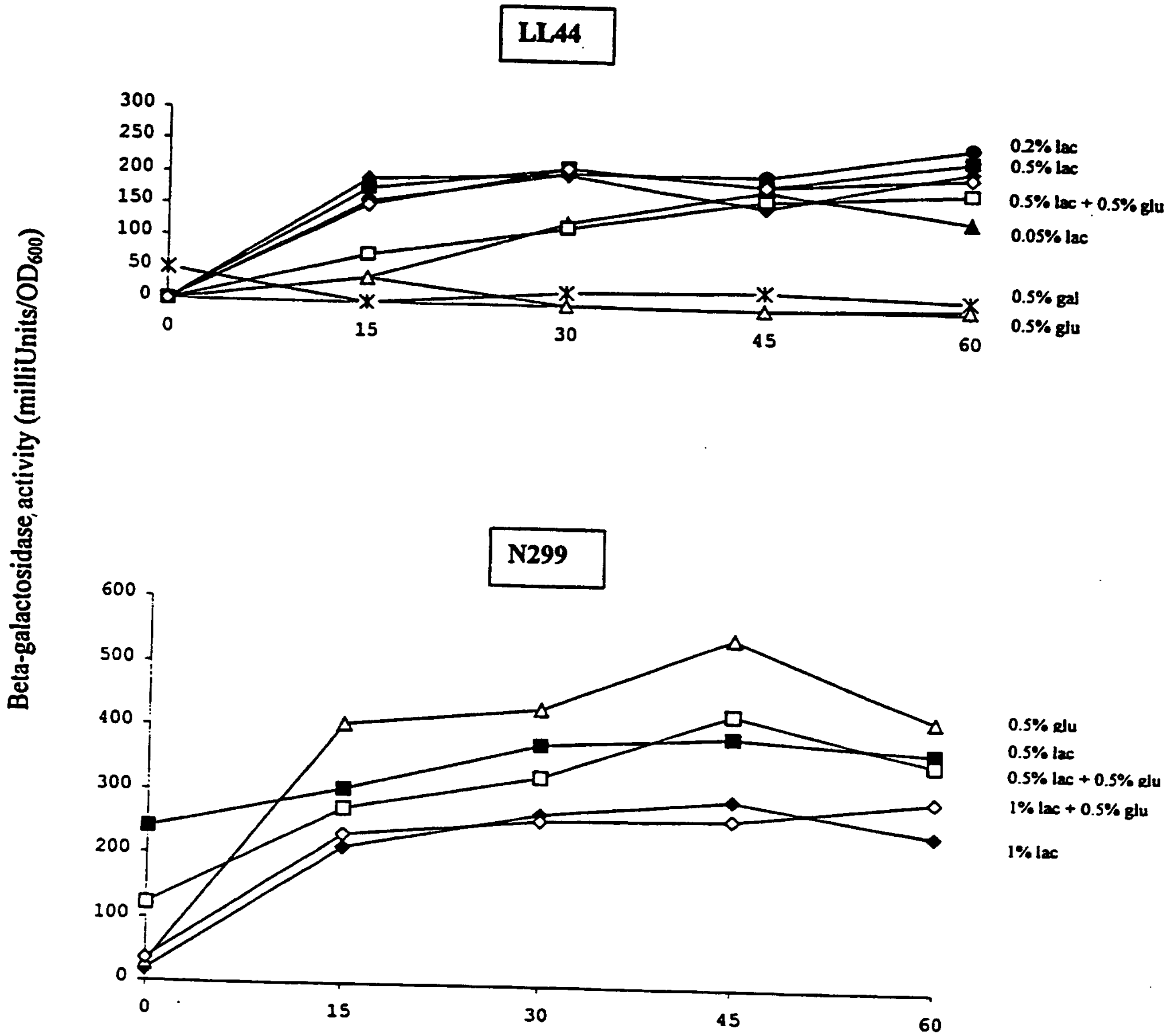

Figure 10: Beta-galactosidase activity (mean of three experiments) of *Lactobacillus delbrueckii* subsp. *lactis* LL44 and *L. delbrueckii* subsp. *bulgaricus* N299. The medium used was BHI-broth containing :
0.5% galactose (✳), 0.05% lactose (▲), 0.2% lactose (●), 0.5% lactose (■), 1.0% lactose (◆), 0.5% glucose (Δ), 0.5% glucose + 0.5% lactose (□), and 0.5% glucose + 1.0% lactose (◇).
Strain N299 did not grow neither in galactose alone nor in 0.05% lactose and the experiment was not realised with these sugars.